Ilorin Journal of Science
Volume 11, Number 2, 2024, pp. 162 – 173 (Printed in Nigeria)
ISSN: 2408 – 4840 © 2024 Faculty of Physical Sciences, University of Ilorin
https://doi.org/10.54908/2024.11.02.005

ILORIN
JOURNAL OF SCEINCE

ILJS-24- 005

# Comparative Study of Phytochemical Profiles and Antioxidant Potential of Leaf and Root Essential Oils from *Clausena anisata* (Willd.) Hook Growing in South-Western Nigeria

**Ismaeel*, R.O. and Usman, L.A.**

Department of Chemistry, University of Ilorin, Ilorin, Nigeria.

## Abstract

The use of synthetic antioxidants to curtail the menace of oxidative stress caused by excessive free radicals in living cells has become worrisome. This is because of the associated drawbacks from their usage and their high costs. It is therefore imperative to provide better antioxidants from natural sources free from side effects. Plant extracts possess biochemicals with proven antioxidant properties. This research therefore explored the comparison of the phytochemicals and antioxidant properties of essential oils from leaves and roots of *Clausena anisata*. This was achieved by subjecting pulverized leaves and roots (500 g each) of the plant to hydrodistillation individually for 4 hours and gave 0.70±0.03 % and 0.43±0.01 % of essential oils respectively. Characterization of the oils was done using GC-MS and the analysis revealed abundance of oxygenated monoterpenoids (46.6% and 23.1%) and oxygenated sesquiterpenoids (20.6% and 36.8%) in the oils. Terpinen-4-ol (24.3% and 6.8%), α-terpineol (13.4% and 11.9%), estragole (9.2% and 5.7%), anethole (6.3% and 6.1%) and α-cadinol (6.1 % and 29.9%) were the major isoprenoids in the oils. The antioxidant activity was investigated by evaluating the oils' abilities to scavenge DPPH radicals in comparison with ascorbic acid. The oils demonstrated moderate DPPH radical scavenging activity with the leaf oil showing a higher activity ($IC_{50}$ = 13.31 µg/mL) than the root oil ($IC_{50}$ = 19.85 µg/mL). Ascorbic acid that was used as a reference showed activity with $IC_{50}$ value of 9.24 µg/mL. These results showed that both oils scavenged DPPH radicals and their activities could be linked to the predominant of oxygenated compounds in the oils. The oils could therefore be used as natural antioxidants to prevent oxidative stress and its health complications after clinical trial.

**Keyword**: *Clausena anisata;* terpinen-4-ol; α-terpineol; α-cadinol; antioxidant

## 1. Introduction

Continuous metabolism of cells produces highly reactive by-products called free radicals. These species are capable of initiating many chain reactions in the body thereby destroying healthy cells and tissues. The human body produces many biological functions including antioxidants which deactivate the free radicals through enzymatic and non-enzymatic pathways before they attack the healthy cells (Ngugi *et al.,* 2019). However, these chemicals, not only produced by human; but also obtained from foods are not enough. Too much of these free radicals in the body results in oxidative stress that plays a significant role in the pathogenesis of many diseases such as ageing, diabetes, liver damage, neurodegeneration, and atherosclerosis among others (Chaves *et al.,* 2020; Garcia-Sanchez *et al.,* 2020; Guchu *et al.,*

Corresponding Author: Ismaeel, R. O.
Email: ismaeel.ro@unilorin.edu.ng

2020; Khan *et al.,* 2012). Oxidative stress and its health implications have been managed using natural antioxidants from plant extracts and their constituents. Although, clinically, the toxicity profile of antioxidants from plant extracts is yet to be thoroughly evaluated and established, they have been reported to be cheaper and safer than synthetic antioxidants (Pokorny, 2007).

The phytochemical constituents of leaf essential oil of *C. anisata* from different parts of the world have been reported. For instance, *E*-ocimenone was the most abundant compound in the leaf oil of the plant native to Cameroon. Other major compounds in the oil include *Z*-ocimenone, γ-terpinene, nerolidol and germacrene D (Tatsadjieub *et al.,* 2008). *β*-Pinene was the most significant compound in the essential oil from leaves of Indian grown *C. anisata*. Pulegone, 1,8-cineole, sabinene and estragole were the other chief constituents in the oil (Venkatesalu, and Senthilkumar, 2009). The leaf oil of the plant from Togo contained estragole in highest quantity compared to other constituents. Other compounds of higher amounts in the oil were trans-anethole, anisaldehyde and *p*-cymene (Nuto *et al.,* 2008). α-Cubebene and anethole were the compounds that existed in greater amounts in the leaf essential oils of *C. anisata* growing in to north-central and south-western parts of Nigeria respectively. The oil of the plant from north-central also had α-pinene, trans-β-pinene, limonene, estragole and caryophyllene in abundant while α-copaene and α-pinene were the other prominent constituents of the south-western grown plant's leaf oil (Lawal *et al*., 2018; Usman *et al.,* 2010).

Rotimi *et al.* (2018), carried out a comparative study on the chemical profiles of essential oils from leaf, root and seed of *Cluasena anisata* native to north-central Nigeria. Variations were observed in the phytochemical profiles of the oils with 8-methylenedispiro[2.1.2.4]undecane, caryophyllene and exaltone being the most abundant compounds in the leaf, root and seed oils respectively. Aromadendrene and germacrene D; β-farnesene, aromadendrene, β-bisabolene and nerolidol and; α-pinene, limonene and β-ocimene were the other major phytochemicals in the oils. The chemical composition of the oil from the leaf of the plant indigenous to south-western Nigeria had earlier been reported [Ekundayo *et al.,* 1987]. Methyl chavicol was the most prominent compound constituting 92.70 % of the oil.

The antioxidant potential of essential oil from leaves of *Clausena anisata* from Cameroon was evaluated by Goudoum *et al.* (2000) using DPPH and β-carotene assays. The oil showed antioxidant activity by scavenging DPPH radicals and prevented β-carotene from undergoing oxidation. The activity was linked to the predominance of estragole, germacrene D, thymol, and E-nerolidol in the oil. Although the leaf essential oil of ancient *C. anisata* in south-west, Nigeria has been documented, as far as we know, the comparative study and antioxidant potentials of leaf and root essential oils of the plant from south-west Nigeria has not been documented. In this context this research was carried out to explore the variations in the chemical constituents and antioxidant potentials of essential oils from leaf and root of *C. anisata native* to south-west, Nigeria.

## 2. Materials and Methods

### 2.1 Collection and Identification of Plant

Fresh roots (2000 g) and leaves of *Clausena anisata* (2300 g) were harvested in Ido-Ijesa, Ilesa, Osun State, Nigeria. The plant was identified at the Herbarium of Plant Biology Department, University of Ilorin, Ilorin, Nigeria and voucher specimens were deposited [UILH/003/0122].

### 2.2 Extraction of Essential Oil

Blended fresh roots (500 g) and leaves (500 g) of *C. anisata* were subjected to hydrodistillation separately for 4 hours in accordance to the specification of British Pharmacopoeia, (1980), and the extractions were done in triplicates. Separately, the oils were collected and conserved in a sealed sample tube and later stored under refrigeration (4 $^{o}$C) until the analyses were performed.

### 2.3 Gas Chromatography–Mass Spectrometry (GC/MS) Characterization of the Oils

A Hewlett–Packard HP 5890A GC, interfaced with a VG analytical 70-250 s double focusing mass spectrometer was used for compound identification in the oils. The GC was equipped with an Orion micromat 412 double-focusing GC system fitted with capillary columns coated with Cp-Sil 5 and Cp-Sil 19 (fused silica, 25 m x 0.25 mm, 0.15 μm film thickness) and flame ionization detector (FID). The volume of each oil injected was 0.2 mL at a split ratio of 1:30. The carrier gas was hydrogen and the oven temperature were programmed from 50 to 230 $^{o}$C at a rate of 3 $^{o}$C/min. Detector temperature was fixed at 200 $^{o}$C while injection temperature was maintained at 250 $^{o}$C. The peak area percentage was calculated using FID signal of the GC HP-chemstation software.

The operating conditions of the MS were: ionization voltage of 70 eV, ion source and transfer line temperature were maintained at 230 $^{o}$C. An on-line desktop with a computer equipped with disk memory was used to process the acquired MS data from the analysis. The GC peak areas were used to compute the percentage composition of the constituents in the oils.

#### 2.3.1 Identification of Constituents in the Oils

The constituents in the oils were identified by (i) comparing the retention indices (RI) of the compounds that was calculated using a homologous series of n-alkanes ($C_7$–$C_{30}$, Supelco Bellefonte, PA, USA) under identical experimental conditions. This was co-injected with standards and compared with the data from Wiley 275 and NIST 08 libraries (ii) comparing the fragmentation pattern in the mass spectra of each constituent with the data from Wiley 275 and NIST 08 libraries (Adams, 2012; Jennings and Shibamoto, 1980; Joualin and Koenig, 1998). The peak area of the GC (FID response) was used to calculate the relative quantity of each constituent without using a correction factor.

### 2.4 Antioxidant Activity of the Oils

DPPH radical assay was used to test the antioxidant potential of the oils in terms of their hydrogen-donating or radical scavenging abilities as reported by Ilhami, 2009. In the method,

1.50 mL of each oil was separately mixed with 95 % solution of ethanol that contained 1.5 ml of $10^{-4}$ M solution of 2,2-diphenyl-1-picryl-hydrazil (DPPH) at ranging concentrations of 12.5 µg/mL to 200 µg/mL. The mixture was incubated in the dark and shaken thoroughly for 30 minutes while the incubation was done at ambient temperature. The procedure was repeated for the control without the oils. UV-visible spectrophotometer was used to measure the absorbance of the solution at 517 nm. The experiment was done in triplicate and the results were expressed as mean values ± standard deviation. The oil's concentration that was responsible for 50 % radical scavenging activity ($IC_{50}$) was calculated from the graph of percentage inhibition versus the oil's concentration. Ascorbic acid was used as reference. The percentage inhibitions were calculated using the equation:

$$\% \text{ Inhibition } = \frac{A_{0-}A_T}{A_0} \times 100 \quad (1)$$

where $A_0$ is the absorbance of the control (sample containing all reagents except the oils) and $A_T$ is the absorbance of the oils.

### 2.5 Statistical Analysis

The tests were run in triplicates and the results were used to calculate the mean values. The data for the biochemical parameters was expressed as mean ± SD (n = 3) and compared using a one-way analysis of variance (ANOVA) test, followed by Dunnett multiple comparison tests with an equal sample size test. Values were considered statistically significant at $p < 0.05$. The non-linear regression analysis from the mean values was used to calculate the $IC_{50}$ values. Statistics were done using SPSS for Windows version 10.

## 3. Result and Discussion

### 3.1 Yields of Essential Oils

The quantities of essential oils afforded by hydrodistillation of the leaves and roots of *C. anisata* were 0.70±0.03 % and 0.43±0.01 % respectively. The yields of essential oils obtained from this study compared favourably with the yields of oils earlier reported from other parts of Nigeria and Cameroon. Previously, Ekundayo *et al.,* 1987, reported higher yield of essential oil from the leaf of the plant from another part of south-western Nigeria than the yield obtained from this study. The differences in the oil yields may be attributed to differences in the environmental conditions of the two locations in different regions of south-western Nigeria. Meanwhile, lower oil yields were obtained from the leaf of the plant growing in Cameroon and north-central Nigeria (Tatsadjieub *et al.,* 2008; Usman *et al.,* 2010). Interestingly, more oils were obtained from the leaf and root of the plant growing in north-central Nigeria in the later report than the yields of oils in this study (Rotimi *et al.,* 2018). The differences in agroclimatic conditions may be responsible for the disparity in the quantity of essential oils extracted as a result of differences in the number of secretory cells in various parts at different locations of the plants.

**Table 1:** Chemical Composition (%) of Root and Leaf Essential Oils of *C. anisata*

| S/N | Compound | % Composition | | RI[a] | RI[b] | Mass spectra data |
|---|---|---|---|---|---|---|
| | | Leaf | Root | | | |
| 1 | α-Pinene | 3.2 | 1.7 | 939 | 933 | 135,105, **93**, 67, 55 |
| 2 | β-Myrcene | - | 2.0 | 991 | 990 | 136,107, **93**, 79, 69 |
| 3 | α –Phellandrene | 1.0 | 3.5 | 1005 | 1007 | 41, 69, **93**, 105, 136 |
| 4 | D-limonene | 0.2 | 2.8 | 1031 | 1027 | 136,107, 93, **68**, 53 |
| 5 | Fenchone | - | 2.0 | 1094 | 1089 | 41, 69, **81**, 109, 152 |
| 6 | Linalool | 1.8 | 5.4 | 1098 | 1098 | 136, 121, **93**, 71,55 |
| 7 | Camphor | 7.1 | - | 1143 | 1140 | 55, 69, 81, **95**, 152 |
| 8 | **Terpinen-4-ol** | **24.3** | 6.8 | 1177 | 1175 | 136, 111, 93, **71**,51 |
| 9 | α-Terpineol | 13.4 | 11.9 | 1189 | 1188 | 136, 121, 93, **59**,51 |
| 10 | Estragole | 9.2 | 5.7 | 1195 | 1197 | 41, 63, **77**, 91, 105 |
| 11 | α-Copaene | - | 2.9 | 1221 | 1221 | 93,105,119,133,147 |
| 12 | Anethole | 6.3 | 6.1 | 1235 | 1234 | 77, 91, 117, 121, **148** |
| 13 | Pulegone | 2.2 | - | 1237 | 1237 | 53, 67, **81**, 97, 112 |
| 14 | Thymol | 1.1 | 3.9 | 1290 | 1290 | 150, **135**,107,91,77 |
| 15 | α-Cubebene | 2.5 | - | 1345 | 1351 | 105,119,**161**,189,204 |
| 16 | β-Caryophyllene | 5.5 | - | 1418 | 1418 | 69,93,105,133, 204 |
| 17 | Aromadendrene | 0.1 | 3.8 | 1461 | 1460 | 69,107,147,**161**,204 |
| 18 | Germacrene D | - | 3.8 | 1471 | 1471 | 204, 136,**121**,93,79 |
| 19 | γ-Cadinene | 0.9 | - | 1513 | 1520 | 55, 91, 119, **161**, 204 |
| 20 | Nerolidol | 7.7 | - | 1564 | 1564 | 41,**69**,93,123,161 |
| 21 | Globulol | 3.0 | 3.6 | 1576 | 1582 | 204, 161,111,**93**,67 |
| 22 | Spathulenol | 1.7 | - | 1578 | 1575 | 220, 147, **119**, 91, 69 |
| 23 | Caryophyllene epoxide | 0.4 | Tr | 1581 | 1580 | 220, 109, 79, 69, 41 |
| 24 | **α-Cadinol** | 6.1 | **29.9** | 1642 | | 55, 121, **161**, 189,222 |
| 25 | β-bisabolol | - | 1.2 | 1668 | 1662 | 69,**82**,93,111,119 |
| 26 | α-Bergamotol | 0.6 | 0.8 | 1693 | 1691 | 43,79,**93**,132,187 |
| 27 | Umbelliferone | 1.1 | 1.1 | 1835 | 1834 | 43, 78, 105, 134, **162** |
| | ***Classes of Compounds*** | | | | | |
| | *Monoterpenoids (Hydrocarbons)* | 4.4 | 10.0 | | | |
| | *Monoterpenoids (Oxygenated)* | 46.6 | 23.1 | | | |
| | *Sesquiterpenoids (Hydrocarbons)* | 9.0 | 10.5 | | | |
| | *Sesquiterpenoids (Oxygenated)* | 20.6 | 36.8 | | | |
| | *Phenylpropanoids* | 18.8 | 18.7 | | | |
| | **Total (%)** | **99.4** | **99.1** | | | |

***Phytochemicals are listed in the order in which they are eluted from fused silica capillary column coated on CP-Sil 5; RI[a] = Literature Retention Indices, RI[b] = Calculated Retention Indices*, Tr = Constituents present in trace amounts (< 0.1 %); Bolded name = Chemotype**

### 3.2 Chemical Composition of the Oils

The identities, percentage composition, and retention indices of the phytochemicals in the leaf and root essential oils of *C. anisata* are presented in Table 1.

From Table 1, twenty-two (22) and twenty (20) compounds with relative percentages of 99.4 % and 99.1 % were detected from the leaf and root essential oils respectively. Oxygenated monoterpenoids (4.6 % and 23.1 %) and oxygenated sesquiterpenoids (20.6 % and 36.8 %) were the chief constituents of the oils. Hydrocarbon monoterpenoids constituted 4.4 % and 10.0 % of the oils. The proportions of hydrocarbon sesquiterpenoids were 9.0 % and 10.5 % in the leaf and root oils respectively. Both oils also contain phenyl propanoids (18.8 % and 18.7 %). Terpinen-4-ol (23.4 % and 6.8 %), α-terpineole (13.4 % and 11.9 %), estragole (9.2 % and 5.7 %), anethole (6.3 % and 6.1 %), α-cadinol (6.1 % and 29.9 %), and globulol (3.0 % and 3.6 %) were the major compounds in the oils. Other chief phytochemicals in the leaf oil comprise α-pinene (3.2 %), camphor (7.1 %), β-caryophyllene (5.5 %), and nerolidol (7.7 %). α-Phellandrene (3.5 %), linalool (5.4 %), thymol (3.9 %), aromadendrene (3.8 %), germacrene D (3.8 %) and D-limonene (2.8 %) were the other compounds that were identified in higher amounts in the root oil.

Phytochemicals that were detected in considerable amounts in the oils were α-bergamotol (0.6 % and 0.8 %) and ubelliferone (1.1 % each). Spathulenol (1.7 %), γ-cadinene (0.9 %), α-cubebene (2.5 %), thymol (1.1 %), pulegone (2.2 %), linalool (1.8 %) and α-phellandrene (1.0 %) were compounds identified in significant quantities in the leaf oil. The root oil also contains α-pinene (1.7 %), β-myrcene (2.0 %), fenchone (2.0 %), and β-bisabolol (1.2 %) in significant quantities. D-limonene (0.2 %), aromadendrene (0.1 %) and caryophyllene epoxide (0.4 %) were obtained in minor quantities in the leaf oil while caryophyllene epoxide was present in trace amount in the root oil.

Since the root and leaf oils contained abundance of α-cadinol and terpinen-4-ol, the oils were therefore of α-cadinol and terpinen-4-ol chemotypes. Methyl chavicol chemotype was previously reported by Ekundayo *et al.,* 1987, in the leaf oil of *C. anisata* from south-western Nigeria. Interestingly, the leaf oil of the plant from north-central Nigeria contained trans-β-ocimene as the chemotype (Usman *et al.,* 2010). In another report, β-caryophyllene was documented as the chemotype of root essential oil of *C. anisata* indigenous to north-central Nigeria (Rotimi *et al.,* 2018). The chemotypic variation could be attributed to the terpene synthases whose activities are determined by environmental conditions due to differences in the plant's geographical locations.

Qualitative and quantitative differences were observed in the composition pattern of essential oils from both parts of the plant. These variations can be associated with the activity of synthases of the most abundant isoprenoids in an essential oil that had been documented to facilitate the conversion of isoprenyl pyrophosphates to various terpenic compounds of structural heterogeneity such as monoterpenoids and sesquiterpenoids in plants. The biosynthetic route occurs via cationic intermediate mechanisms followed by deprotonation or hydration of the intermediate cations to terminate the reaction (Bohlmann *et al.,* 1997; Chen *et*

*al.,* 2016; Davis and Croteau, 2000; Degenhardt *et al.,* 2009; Dudareva *et al.,* 2003; Karunanithi and Zerbe, 2019; Usman and Ismaeel, 2020). Therefore, the synthases of terpinen-4-ol and β-caryophyllene in the leaf oil as well as α-terpineol and α-cadinol synthases in the root oil catalysed the formation of all the isoprenoids in the oils. Environmental factors and/or agroclimatic conditions have been established as factors that dictate the activity of terpene synthases that eventually influence the type and quantities of essential oil constituents (Qjang *et al.,* 2018; Usman *et al.,* 2022; Verma and Shukla, 2015). This is evidenced in the phytochemical profiles of the root and leaf essential oils obtained in this study. The synthase of terpinen-4-ol was more active than that of β-caryophyllene and mediated the biosynthesis of more monoterpenoids (51.0 %) than sesquiterpenoids (29.6 %) in the leaf oil. Meanwhile, sesquiterpenoids (47.3 %) were more abundant in the root oil compared to monoterpenoids (33.1 %). This indicated that α-cadinol synthase was more active than α-terpineol synthase in the root of *C. anisata*.

α-Terpineol and terpinen-4-ol synthases facilitated the conversion of geranyl, linalyl and neryl pyrophosphates to β-pinene, terpinen-4-ol, α-phellandrene, α-terpineol, linalool, and D-limonene in the leaf and root oils of *C. anisata*. Meanwhile, terpinen-4-ol, α-pinene, and α-terpineol were present in higher amounts in the oil of the leaf oil than the root oil. On the contrary, the quantities of D-limonene, linalool and α-phellandrene were higher in the root oil than in the oil from the leaf. Similarly, β-caryophyllene and α-cadinol synthases mediated the conversion of farnesyl and nerolidyl pyrophosphates to aromadendrene, globulol, caryophyllene epoxide, α-cadinol, α-bergamotol and umbelliferone via cationic reaction mechanisms in the oils. However, β-caryophyllene synthase was more active and aided the biosynthesis of caryophyllene epoxide in higher amount in the leaf oil than α-cadinol synthase, which facilitated the biogenesis of the caryophyllene oxide in lower quantity in the root oil. Equivalently, the amounts of α-cadinol and globulol in the root oil were greater than their quantities in the leaf oil. The different quantities of the aforementioned compounds could be linked to the physiological conditions of the plant that varied in the leaf and root. These differences are determined by agroclimatic conditions that also alter the activity of the isoprenyl synthases in plant (Pattanaik and Lindberg, 2015).

Qualitatively, terpinen-4-ol synthase mediated the formation of camphor in the leaf oil. Surprisingly, the compound was not detected in the root oil. Comparably, β-myrcene and fenchone, whose biosyntheses were aided by α-terpineol synthase in the root oil, were unidentified in the leaf oil. Like wisely, α-cubebene, β-caryophyllene, nerolidol, γ-cadinene and spathulenol which were biosynthesized in the leaf oil via β-caryophyllene synthase mediated cationic reactions were not detected in the root oil. Correspondingly, α-cadinol synthase facilitated the formation of α-copaene, germacrene D and β-bisabolol in the root oil. Meanwhile, the compounds were not found in the leaf oil. The qualitative variation of phytochemicals in the oils revealed that the activities of the isoprenoid synthases was affected by the physiological conditions of both parts of the plant.

### 3.3 DPPH Radical Scavenging Activity of the Oils

The ability to scavenge DPPH radicals was used to evaluate the oils' antioxidant activity. The oils were active as antioxidant and the activity varied with concentration. There was a steady increase in the activity of the leaf oil from 40.3±0.01 % to 82.2±0.02 % and that of the root oil from 43.2±0.01 % to 77.4±0.01 % as concentration ranges from 2.5 to 200 µg/mL [Figure 1]. The concentrations that resulted in 50 % radical scavenging activity ($IC_{50}$) were 13.31 µg/mL and 19.85 µg/mL for the leaf and root oils respectively. Ascorbic acid gave an $IC_{50}$ value of 9.24 µg/mL.

The correlation between the antioxidant capacity of essential oils and their phytochemicals has been studied and reported by many authors. For instance, the synergistic effect of thymol and linalool, which are hydroxyl group-containing constituents of seed essential oil of *Satureja macrostema* was reported to be accountable for the antioxidant activity of the oil (Torres-Martinez *et al.,* 2018). In addition, the antioxidant capacity of carvacrol, thymol, hydroxytyrosol, zingerone and 6-gingerol were separately investigated. The compounds reportedly exhibited antioxidant property with thymol showing the highest activity (Aeschbach *et al.,* 1994). However, essential oils from *T. caespititius*, *T. camphorates* and *T. mastichina* contained linalool in high amounts with no trace amount of thymol. The oils exhibited greater antioxidant activity that was linked to the abundant of linalool (Miguel *et al.,* 2004). This was similar to the result obtained by Zuo *et al.*, 2021, in which a high linalool-containing essential oil of *Cinnamomum camphora* (L.) showed very high antioxidant activity compared to the activity of other essential oils with high amounts of eucalyptol, camphor and borneol from the same plant. The antioxidant activity of terpinen-4-ol, α-terpineol, nerolidol and globulol was investigated and documented (Bicas *et al.,* 2011; Ojha *et al.,* 2021; Rodriguez *et al.,* 2009; Souza *et al.,* 2018). In this study, the leaf oil was better as an antioxidant than the root oil. The higher antioxidant potential of the leaf oil could therefore be attributed to the higher quantities of terpinen-4-ol, α-terpineol, nerolidol, globulol, estragole, pulegone and camphor as compared to their quantities in the root oil.

## 4. Conclusion

The phytochemical profile and antioxidant potential of the volatile oils from the leaf and root of *C. anisata* were successfully evaluated and compared. The oils showed variations in their phytochemical profiles. β-Caryophyllene and terpinen-4-ol were the most prominent constituents of the leaf oil while α-terpineol and α-cadinol were the monoterpenoids and sesquiterpenoids of the highest quantities in the root oil. The oils displayed antioxidant activity at all tested concentrations by scavenging DPPH radicals. Meanwhile, the leaf oil showed greater activity than the root oil. The higher activity was associated to the greater amounts of some oxygenated compounds in the leaf oil than the quantities of the same phytochemicals in the root oil. Although ascorbic acid was more active than both oils, the oils could be used as natural antioxidants to prevent oxidative stress and other clinically related diseases after their toxicity has been established.

**Acknowledgement**

The authors acknowledged Mr. Bolu of the Herbarium of Department of Plant Biology, University of Ilorin, Ilorin, Nigeria who assisted in the identification of the plant and documentation of voucher specimens.

**References**

Adams, R.P. (2012): Identification of essential oil components by gas chromatography and mass spectrometry. Allured publ. corp., Carol Stream, IL. USA pp. 112 ISBN: 978-1-932633-21-4

Aeschbach, R., Loliger, J., Scott, B.C., Murcia, A., Butler, J. and Halliwell, B. (1994):
Antioxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. *Food Chem. Toxicol*. **32**: 31 – 36. https://doi.org/10.1016/0278-6915(84)90033-4

Bicas, J.L., Neri-Numa, I.A., Ruiz, A.L.T.G., De Carvalho, J.E. and Pastore, G.M. (2011): Evaluation of the antioxidant and antiproliferative potential of bioflavols. *Food Chem. Toxicol*. **49**(7), 1610 – 1615. https://doi.org/10.1002/bbb.2320

Bohlmann, J., Steel, C.L. and Croteau, R. (1997): Monoterpene synthases from grand fir (*Abies grandis*) cDNA isolation, characterization, and functional expression of myrcene synthases, (-)-(4S)-limonene synthase and (-)-(1S,5S)-pinene synthase. *J. Bio. Chem.* **272**: 21784 – 21792.

British pharmacopoeia II. H. M., stationery office, London, (1980). pp. 109

Chaves, N., Santiago A., and Alias, J. C. (2020): Quantification of antioxidant activity of plant extracts: Analysis of sensitivity and Hierarchization based on the method used. *Antioxidants*. **9**(1), 76. doi: 10.3390/antiox9010076

Chen, X., Köllner, T.G., Jia, Q., Norris, A., Santhanam, B., Rabe, P., Jeroen, S.D., Gad, S., Jonathan, G. and Feng, C. (2016): Terpene synthase genes in eukaryotes beyond plants and fungi: occurrence in social amoebae. *Proc. Natl. Acad. Sci*. 113, 12132–12137. doi:10.1073/pnas.1610379113

Davis, E.M. and Croteau, R. (2000): Cyclization enzymes in the biosynthesis of monoterpenes, sesquiterpenes, and diterpenes," in *Biosynthesis*. *Topics Current Chem.* vol. 209 Eds. Leeper F. J., Vederas J. C. (Berlin, Heidelberg: Springer) pp. 53–95

Degenhardt, J., Ko¨llner, T.G. and Gershenzon, J. (2009): Monoterpene and sesquiterpene synthases and the origin of terpene skeletal diversity in plants. *Phytochem*. **70**, 1621–1637. doi: 10.1016/j.phytochem.2009.07.030

Dudareva, N., Martin, D., Kish, C.M., Kolosova, N., Gorenstein, N., Faldt, J., Miller, B. and Bohlmann, J. (2003): (E)-beta-ocimene and myrcene synthase genes of floral scent biosynthesis in snapdragon: function and expression of three terpene synthase genes of a new terpene synthase subfamily. *Plant Cell*. **15**(5), 1227 – 1241. doi: 10.1105/tpc.011015

Ekundayo, O., Oguntimein, B.O. and Hammerschmidt, F.J. (1987): Constituents of the essential oil of *Clausena anisate* leaves. *Planta Medica*. **52**(06), 505 – 506. doi:10.1055/s-2007-969274

Garcia-Sanchez, A., Miranda-Diaz, A.G. and Cardona-Munoz, E.G. (2020): The roles of oxidative stress in physiopathology and pharmacological treatment with pro- and antioxidant properties in chronic diseases. *Oxid. Med. Cell. Longev*. **2020**, 1–15 https://doi.org/10.1155/2020/2082145

Goudoum, A., Tinkeu, L.S.N., Ngassoum, M.B. and Mbofung, C.M. (2000): Antioxidant activities of essential oils of *Clausena anisata* (Rutaceae) and *Plectranthus glandulosus* (Labiateae), plants used against stored grain insects in North Cameroon. *Int. J. Biochem. Sci*. **3**(3), 567 – 577. doi:10.4314/ijbcs.v3i3.45338

Guchu, B.M., Machocho, A.K., Mwihia, S.K. and Ngugi, M.P. (2020): In vitro antioxidant activities of methanolic extracts of *Caesalphinia volkensii* Harms., *Vernonia lasiopus* O. Hoffm., and *Acacia hockii* De Wild. *J. Evid.-Based. Complem. Altern. Med*. **1**, 1–10. doi: 10.1155/2020/3586268

Ilhami, G. (2009): Antioxidant activity of L-adrenaline: A structure-activity insight. *Chem-Biol. Interact*. **179**(2-3), 71-80. https://doi.org/10.1016/j.cbi.2008.09.023

Jennings, W. and Shibamoto, T. (1980): Qualitative analysis of flavour volatiles by gas capillary chromatography. Academic Press, New York pp. 68 – 109

Joulain, D. and Koenig, W. A. (1998): The atlas of spectra data of sesquiterpene hydrocarbon. E.B. Verlay Hamburg, Germany pp. 112 – 153

Karunanithi, P.S. and Zerbe, P. (2019): Terpene synthases as metabolic gatekeepers in the evolution of plant terpenoid chemical diversity. *Front Plant Sci*. **10**(1166), 1–23. doi: 10.3389/fpls.2019.01166

Khan, R.M., Saeed, N. and Shabbir, M. (2012): Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L., *BMC Complement. Med. Ther*. **12**, 221. doi: 10.1186/1472-6882-12-221

Lawal, I.O., Ajay, H., Dairo, P. and Ogunbamowo, P.O. (2018): Comparative studies of essential oils of Clausena anisata (Hook) using solvent-free microwave extraction and hydrodistillation methods. *JOMPED*. **3**(1), a59. doi: https://doi.org/10.4102/jomped.v3i1.59

Miguel, G., Simoes, M., Figueiredo, A.C., Barroso, J.G., Pedro, L.G. and Carvalho, L. (2004): Composition and antioxidant activities of essential oils of *Thymus caespititius*, *Thymus camphorates* and *Thymus mastichina*. *Food Chem*. **86**, 183–188. https://doi.org/10.1016/j.foodchem.2003.08.031

Ngugi, M.P., Arika, W., Kibiti, C.M. and Njagi, J.M. (2019): In vitro antioxidant properties of dichloromethanolic leaf extract of *Gnidia glauca* (Fresen) as a promising antiobesity drug. *J. Evid-Based. Integr. Med*. **24**, 1-17. doi: 10.1177/2515690X19883258

Nuto, Y., Mollong, R., Kasseney, D.B. and Gomina, M.J. (2008): Acaricidal effect of essential oil of *Clausena anisata* (Rutaceae) on larvae of three tick species: *Amblyomma variegatum*, *Rhipicephalus* (Boophilus) *decoloratus* and *Rhipicephalus* (Boophilus) *microplus*. *Entomol. Nematol*. **10**(4), 23 – 34. doi: 10.5897/JEN2018.0203

Ojha, S., Arunachalam, S., Meeran, M.F.N., Azimullah, S., Sharma, C. and Sameer, N.G. (2021): Nerolidol attenuates oxidative stress, inflammation, and apoptosis by modulating Nrf2/MAPK signalling pathways in doxorubicin-induced acute cardiotoxicity in rats. *Antioxidants* **10**(6)c: 984. doi: 10.3390/antiox10060984

Pattanaik, B. and Lindberg, P. (2015): Terpenoids and their biosynthesis in cyanobacteria. *Life*. **5**(1), 269 – 293. doi: 10.3390/life5010269

Pokorny, J. (2007): Are natural antioxidants better – and safer – than synthetic antioxidants? *Eur. J. Lipid Sci. Technol*. **109**(6), 629 – 642. doi: 10.1002/ejlt.200700064

Qjang, W., Li, Y, Kui-Shan, W., Xiao, R., Ying-Xiang, Z. and Feng, W. (2018): Response of plant secondary metabolites to environmental factors. *Mol*. **23**(4), 762. doi: 10.3390/molecules23040762

Rodriguez, S., Sueiro, R.A., Murray, P.A. and Leiro, J.M. (2009): Bioactive sesquiterpene obtained from *Schnius areira* L. (Anacardiaceae) essential oil. *Proceedings*. **41**, 85. https://doi.org/10.3390/ecsoc-23-06649

Rotimi, L., Okoh, O.O., Ilecholubo, A.P. and Okoh, A.I. (2018): Comparative study of chemical profiles of leaf, root and seed essential oils of *Clausena anisata* (Willd.) Hook. *Asian J. Chem*. **30**(3), 487 – 490. doi: 10.14233/ajchem.2018.20867

Souza, C.F., Baldissera, M., Silva, L.L., Geihs, M.A. and Baldisserotto, B. (2018): Is monoterpene terpinen-4-ol the compound responsible for the anaesthetic and antioxidant activity of *Melaleuca alternifolia* essential oil (tea tree oil) in silver catfish? *Aquaculture*. **486**, 217 – 223. https://doi.org/10.1016/j.aquaculture.2017.12.025

Tatsadjieub, L.N., Yaoubaa, A., Dongmoac, P.M.J., Etoad, F.X., Mbofunga, C.M.F., Zollo, P.H.A. and Menut, C. (2008): Evaluation of *Clausena anisata* essential oil from Cameroon for Controlling food spoilage fungi and its potential use as an antiradical agent. *Nat. Prod. Commun.* **6**(9), 1367 – 1371. https://doi.org/10.1177/1934578X1100600937

Torres-Martinez, R., Garcia-Rodriguez, Y.M., Rios-Chavez, P., Saavedra, A., Lopez-Meza, J. E., Ochoa-Zarzosa, A. and Garciglia, R.S. (2018): Antioxidant activity of the essential oil and its major terpenes of *Satureja macrostema* (Moc. And Sesse ex Benth.) Briq. *Pharmacogn. Mag.* S875 – S880. doi: 10.4103/pm.pm_316_17

Venkatesalu, V. and Senthilkumar, A. (2009): Phytochemical analysis and antibacterial activity of the essential oil of Clausena anisata (Willd.) Hook. f. ex Benth. *Int. J. Integr. Biol*. **5**(2), 116 – 120.

Verma, N. and Shukla, S. J. (2015): Impact of various factors responsible for fluctuation in plant secondary metabolites. *Appl. Res. Med. Aromat. Plants*. **2**, 105–113. https://doi.org/10.1016/j.jarmap.2015.09.002

Usman, L.A., Hamid, A.A., Olawore, S.O., Fakunle, C.O., Oladosu, I.A. and Zubair, M.F. (2010): Chemical composition of *Clausena anisate* grown in north-central Nigeria. *J. Agric. Biol. Sci*. **6**(6), 891 – 894.

Usman, L.A. and Ismaeel, R. O. (2020): Chemical Composition of Root Essential oil of *Peperomia pellucida* (L.) Kunth. Grown in Nigeria. *J. Essent. Oil-Bear. Plants*. **23**(3), 628 – 632.

Usman, L.A., Oguntoye, O.S. and Ismaeel, R.O. (2022): Phytochemical profile, antioxidant and antidiabetic potential of essential oil from fresh and dried leaves of *Eucalyptus globulus. Journal of the Chilean Chemical Society. **67**(1), 5453 – 5461.*

Zuo, Z., Luo, Q., Tian, Z., Zheng, T., Xu, S., Ma, Y. and Zuo, S. (2021): Terpenoid composition and antioxidant activity of extracts from four chemotypes of *Cinnamomum camphora* and their main antioxidant agents. *Biofuels Bioprod. Biorefin.* **16**(2), 510–522. https://doi.org/10.1002/bbb.2320